

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: [D405, JY410, T542, D543, K593, Y595, Y385, G387, and G388.
2. (Original) The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
3. (Currently Amended) The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase [Deep Vent DNA polymerase], Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase (SEQ ID NO. 10), PGB-D DNA polymerase (Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.
4. (Previously Cancelled) The enzyme mixture of claim 1, wherein said second enzyme is a mutant DNA polymerase.
5. (Previously Cancelled) The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.
6. (Currently Cancelled) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
7. (Previously Cancelled) An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is [a Taq]Archaeal DNA polymerase,

said second enzyme is a mutant [Pfu]Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

8. (Previously Cancelled) The enzyme mixture of claim [4]Z, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

9. (Currently Cancelled) The enzyme mixture of claim 6, wherein said mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

10. (Currently Amended) The enzyme mixture of claim 1[or 9], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: [D405E,]Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

11. (Previously Amended) The enzyme mixture of claim 1, further comprising a PCR enhancing factor and/or an additive.

12. (Currently Amended) A kit comprising a first enzyme, a second enzyme, and packaging material therefor, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: [D405,]Y410, T542, D543, K593, Y595, Y385, G387, and G388.

13. (Original) The kit of claim 12, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

14. (Currently Amended) The kit of claim 13, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo[Deep Vent]DNA polymerase, Tgo DNA

polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase (SEQ ID NO. 10), PGB-D DNA polymerase(Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.

15. (Previously Cancelled) The kit of claim 14, wherein said second enzyme is a mutant DNA polymerase.

16. (Previously Cancelled) The kit of claim 15, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

17. (Previously Cancelled) The kit of claim 16, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

18. (Currently Cancelled) A kit comprising an enzyme mixture comprising a first enzyme and a second enzyme, and packaging material therefor, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

19. (Previously Cancelled) A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a Taq DNA polymerase, and packaging material therefore, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

20. (Currently Amended) The kit of claim 12[, or 18], further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

21. (Currently Cancelled) The kit of claim 18, wherein said mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid

positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

22. (Currently Amended) The kit of claim 12[or 21], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: [D405E, JY410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

23. (Previously Withdrawn from Consideration) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

24. (Previously Withdrawn from Consideration) The method of claim 23, wherein said nucleic acid template is a DNA [or an RNA] molecule.

25. (Previously Withdrawn from Consideration) The method of claim 24, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

26. (Previously Withdrawn from Consideration) The method of claim 25, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

27. (Previously Cancelled) The method of claim 24, wherein said second enzyme is a mutant DNA polymerase.

28. (Previously Cancelled) The method of claim 27, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UITma DNA

polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

29. (Previously Cancelled) The method of claim 27, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

30. (Previously Withdrawn from Consideration) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

31. (Previously Withdrawn from Consideration) A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

32. (Previously Withdrawn from Consideration) The method of claim [28,]30, or 31, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

33. (Previously Withdrawn from Consideration) The method of claim 23 [32], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group

consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

34. (Previously Withdrawn from Consideration) The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

35. (Previously Withdrawn from Consideration) The method of claim 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

36. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: [D405,]Y410, T542, D543, K593, Y595, Y385, G387, and G388.

37. (Currently Amended) The enzyme mixture of claim 36, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: [D405E,]Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

38. (Previously Added) The enzyme mixture of claim 36, wherein said mutant Pfu DNA polymerase comprises a mutation at amino acid position G387.

39. (Previously Added) The enzyme mixture of claim 36, wherein said mutant Pfu DNA polymerase comprises a mutation of G387P.

40. (Previously Added) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a KOD DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

41. (Previously Added) The enzyme mixture of claim 40, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

42. (Previously Added) The enzyme mixture of claim 40, wherein said mutant Pfu DNA polymerase comprises a mutation at amino acid position G387.

43. (Previously Added) The enzyme mixture of claim 40, wherein said mutant Pfu DNA polymerase comprises a mutation of G387P.

44. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase (SEQ ID NO. 10), and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

45. (Previously Added) The enzyme mixture of claim 44, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

46. (Previously Added) The enzyme mixture of claim 44, wherein said mutant Pfu DNA polymerase comprises a mutation at amino acid position G387.

47. (Previously Added) The enzyme mixture of claim 44, wherein said mutant Pfu DNA polymerase comprises a mutation of G387P.

48. (Currently Amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: [D405,]Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor.

49. (Previously Added) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a KOD DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor.

50. (Currently Amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase (SEQ ID NO. 10), and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor.

51. (Previously Added) The kit of claim 48, 49, or 50, wherein said kit further comprises a reagent selected from the group consisting of: dNTPs, reaction buffer, primer, and DNA enhancing factor.